Biocides and other Harmful Substances on Dry and Fluid Preserved Specimens

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Abstract Biocide-contaminated dry specimens and harmful substances in fluid preserved specimens can be found in many scientific museums and collections. Early preservation methods of organic tissue relied on using mainly essential oils and salts for the manufacture of human and animal mummies. In the last 500 years, many technical improvements have been introduced for preserving specimens for scientific research. Through the use of arsenic trioxide in the course of the 18th and 19th centuries it became possible to preserve animal skins more effectively. This substance, among others, persisted as a biocide until the 20th century. By then, many biocides were developed, especially pesticides that were used for the industrial purposes. Many institutions began to use a large variety of these new biocides to preserve their collections. Preparation technicians included some of these in the manufacture of specimens. Therefore, the conservation and care of such cultural assets might be associated with a health risk. This article draws attention to the problem providing a historical overview of the various poisons used in preparation technology. It also presents some methods for analysis and occupational safety suggestions. At the same time, it demonstrates the importance of good networking among the various professional groups and the resulting interdisciplinary cooperation.

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For the last few centuries, the only method to preserve *natural history and anatomical collections*[1] from decay was using hazardous preservation agents (**Tab. 1**). The substances used for this purpose act as biocides to prevent the collected specimens from being infested by pests. Some of the first documented preparation methods for natural history collections were derived from the mummification techniques of ancient Egypt, in which dead bodies were dried and treated with salt, essential oils and certain essences.[2] During the first wave of European colonisation, these practices gained popularity in the preservation of bird skins and other organic tissue shipped into Europe from the newly seized colonies around the world.[3] Pharmaceutical knowledge played a very important role in the history of preservation. Many natural history specimens are found in the collections of naturalists and apothecaries of the 16th century.[4]

With the technical improvement of preparation methods and the knowledge gained from pharmacology and chemistry, the quality of the preserved specimens was significantly increased. In parallel the implementation of glass plate covers in collection drawers was a major development for a better preservation of the specimens, making it possible to view the collections without taking them out of their display case. This prevented possible damage of the collections through uncautious handling and protected the collection against infestation from bigger pests.[5] These technical innovations simplified the study of nature, which developed intensively at the end of the 17th century. They allowed it on the one hand to illustrate unknown species and on the other hand to preserve the specimens in their entirety for further study and description.[6] It can therefore be considered as an important step for natural sciences, culminating in the founding of the Musée d'Historie Naturelle in Paris. This first natural history museum was founded in 1793 and was the stepping to the long process of institutionalization of natural history collections.[7]

1 There are many different types of specific specimens types including human anatomical/pathological, veterinary anatomical/pathological, zoological and botanical specimens. Here and in the following text these will be simplified as "natural history and anatomical collections".

2 e.g. Jahn 1986, p. 11.

3 ibid. p. 12.

4 Particularly the collections of the botanist Ulysse Aldrovandi (1522–1605), which can be seen today at the Palazzo Poggi Museum in Bologna and the cabinet of natural history from the Neapolitan pharmacist Ferrante Imperato (1550–1615), depicted in the frontispiece of his "Dell'historia naturale" (1599), are very good examples of these first preparation techniques that were developed in the 16th century. Dilg 1994, pp. 457–458.

5 e.g. Siemers 2004, p. 191.

6 e.g. Behrmann 1972, p. 116; Echsel/Ráček 1976, p. 12.; Jahn 1986, p. 14.

7 e.g. Siemers 2004, p. 346.

Tab. 1 Relevant publications on preservation methods for natural history collections since the 16th century (without claim to completeness)

Year	Preservation method	Reference
1555	Salt, essential oils and essences, as well as drying processes. Adaptation from Egyptian mummification techniques transmitted by Herodot (490–425 B.C.E.) and Plinius (23–79 B.C.E.) published in Paris by Pierre Belon (1517–1564).	Behrmann 1972, pp. 111–115 Jahn 1986, pp. 11–12.
1662	First documented use of "spirit" (ethanol) for the preservation of specimens in fluid by William Croone (1633–1684).	Simmons 2014, p. 10.
1743	Preservation of bird skins and specimen mounts with Baptiste Bécœur's (1718–1777) "Arsenical soap": camphor, powdered arsenic, soap, tartaric acid, and powdered quicklime (unpublished and kept a secret until 1800). The mixture was published in Paris in 1800 by Francois-Marie Daudin (1774–1804).	Farber 1977, pp. 560–561.
1751	Recommendation of the use of mercury for sealing specimen jars with ground glass stoppers by Carl Linné (1707–1778). The liquid metal was added to the preservation fluid, the jar was then closed and turned upside down so that the mercury was spread into the spaces between the glass and the stopper.	Linné/Hannoverische Bey-träge 1759, pp. 339–440.
1771	Tesser Samuel Kuckahn's (date of birth is unknown–1776) methods for the preparation of a liquid varnish made of turpentine, camphor, and spirit of turpentine for skin treatment and the preparation of a dry compound made of corrosive sublimate, saltpeter, alum, sulfur, musk, pepper, and ground tobacco to apply on the treated skin. The feet were coated with varnish and the entire mounted specimen was dried in an oven.	Farber 1977, p. 555.
1825	Charles Waterton (1782–1865) recommends treating bird skins with corrosive sublimate dissolved in alcohol before mounting them.	Farber 1977, p. 561 f. 40.
1835	Suggestion by Otto Ernst Oppermann (1764–1851) to put more emphasis on the removal of fats of the preserved specimen, to work cleanly and avoid the use of toxic substances such as arsenic.	Oppermann 1835, p. 272.
1893	First publication of the use of formaldehyde solutions for the fixation and preservation of fluid preserved specimens (commercially sold as Formalin or Formol) by Ferdinand Blum (1865–1959) and his father Isaac Blum (1833–1903).	König/Winter 2020, pp. 13–16.
1924	First documented recommendation to use Eulans for the protection of cultural heritage in Germany.	Homolka 2014(I) p. 68.
1984	Suggestion to use methoxychlor as an alternative to Eulan U33 for the use in taxidermy in eastern European countries.	Spicale 1984, p. 183.

Tab. 1 continued

Year	Preservation method	Reference
1998	5th edition of the most relevant book on preparation technology in Germany "Makroskopische Präparationstechnik. Teil 1: Leitfaden für das Sammeln, Präparieren und Konservieren: Wirbeltiere" by Rudolf Piechocki (1919–2000). This recompilation of preparations still recommended using arsenic trioxide, naphthalene, PDCB and lindane (among others) for the preservation of new preparations and many highly toxic fumigation gases for the treatment of museum collection storage.	Piechocki/Altner 1998, p. 24; pp. 27–28; p. 123; pp. 194–195.
2012	Implementation of the "Biocidal Products Regulation" in which biocides are organized into 4 main groups (disinfectants, protective agents, pesticides and "other biocides") strictly sorting 22 product types, which are categorized depending on their purpose. Product type Nr. 22 (fluids for embalmment and taxidermy – group 4) includes only iodine, leaving all other biocides used in this field as unauthorized products.	Verordnung (EU) Nr. 528/2012, Anhang V, L167/105–107.
2017	Proposal for the production of biocide-free preparations to protect the safety and health of museum professionals caring for natural history collections.	Troxler 2017, p. 43.

Early preparation techniques can be divided in two main categories, either dry preparations such as mummies, skeleton and specimens mounts; or fluid preserved specimens, where the whole specimen is preserved in a fluid that inhibits its decay.[8]

The long-term preservation of dry specimens was only possible by protecting them from pests that feed on organic tissue, specially keratin. Therefore, the use of different hazardous substances was necessary. The so-called "arsenic soap", developed around 1740 by the French pharmacist Jean-Baptiste Bécœur (1718–1777), played a very important role in the field of taxidermy[9]. It was a reliable agent for the preservation of skin, bones and feathers, so it helped to improve the preservation of bird specimens at an early stage. The use of arsenic trioxide became popular after the first publication of this method around 1800 and was used not only as a "soap", but also as a powder.[10] The modifications of Bécœur's method were published throughout Europe in different manuals for the collection of specimens, always relying on the use of arsenic trioxide as

8 e.g. Jahn 1986, pp. 11–30.

9 Taxidermy is the field of zoological preparation that developed around the 19th century and is specialized in the arrangement of lifelike specimen mounts (in German referred to as "Dermoplastik"). e.g. Becker 2004, p. 16.

10 Bécœur's preparation was published by Francois-Marie Daudin (1774–1804) in his "Trait elementaire et complet d'ornithologie, ou histoire naturelle des oiseaux" (1800) and reprinted 20 years later with a modification by Louis Dufresne (1752–1832) in 1820. Farber 1977, pp. 557–561.

the most important poison among other substances.[11] Another preservation agent and harmful substance was corrosive sublimate (mercury bichloride), which was added to some of the mixtures.[12]

Specimens in fluid were preserved mainly through two different principles, either by dehydration or by action of biocides in the fluid. By using a hygroscopic fluid, the water in the specimen is removed and preserves the specimen by dehydration, stopping autolysis and other inherent processes of decay.[13] Biocides in the fluid act as disinfectants which stop possible sources of decay. There are some substances that have both qualities, as in the case of ethanol. But there are many cases, where biocides are added to hygroscopic substances mainly for more effective preservation.[14] To improve these methods the specimen jars were closed with an airtight lid or sealing paste to prevent oxidation processes and evaporation of the preservation fluid.[15] The first documented preservation fluid is known as "spirit" (ethanol) and its first use dates back to the year 1662.[16] Usually it was mixed with spices to enhance its preserving properties. There are some historical preparations of mixtures that also include the use of corrosive sublimate in the preservation fluid.[17]

While the use of arsenic trioxide, mercury chloride and ethanol where the main biocides used for specimen preservation until the 19th century, this changed by the end of that century, when a wide range of different toxic substances were developed during the industrialization era. These where used as insecticides for agriculture, wood products and especially for the textile industry. Some of this substances were considered by museums and adapted for the preservation (or fumigation) of existing collections. Many of them where introduced in preparation techniques for the manufacture of new specimens.[18]

A few of the hazardous substances that were used during the beginning of the 20th century are listed below:[19]

11 A list with some of these publications can be seen in Farber. p. 563 f. 46.

12 e.g. Bacon et al. 2011, p. 111.

13 Simmons 2014, p. 48.

14 As in the case of glycerol is, the main ingredient in the so-called "Kaiserling" and "Jores" preservation solutions. It is very common to ad some biocide in the solution against possible mold growth. e.g. König/Winter 2020, p. 11; p. 27; p. 32.

15 e.g. Linné/Hannoverische Beyträge 1759, pp. 339–440. The old principle of airtight jar sealing is still as important today as it was more than 250 years ago. Meier/Wechsler 2016, p. 17.

16 The English physician and fellow of the Royal Society William Croone (1633–1684) preserved the embryo of a dog for many months and presented it to his peers. Simmons 2014, p. 10.

17 e.g. Simmons et al. 2007, p. 34.

18 e.g. Tello/Paz 2013, p. 7.

19 This list shows many of the preservation substances used by German museum professionals. Piechocki/Altner 1998, pp. 25–30; Tello/Paz 2013, p. 7.

- Napthalene
- Formaldehyde
- Carbolic acid (Phenol)
- PDCB (para-Dichlorobenzene)
- Lindane
- DDT (Dichlorodiphenyltrichloroethane)

The use of these substances enabled more efficient preservation because they seemed to be less hazardous than the old chemicals used. By the end of the 19th century many taxidermists were already aware that arsenic trioxide was known to cause "coughs, colds, chronic bronchitis, soreness of the lips and nose, ugly ulcers, brittleness of nails, and partial or complete paralysis".[20]

Many European countries started to avoid some of these toxic substances after decades of use in agriculture, industry and health care because they showed a risk to health and the environment. The best-known prohibitions were imposed on substances such as arsenic trioxide, mercury compounds and DDT.[21] This made it very difficult for museums to access these substances and protect exhibits from pests.

The wide use of arsenic trioxide in taxidermy stopped around the end of the 20th century.[22] It was replaced by pyrethroids and a group of insecticides known as Eulans.[23] They became increasingly popular by targeting only specific pests. Eulans were originally developed for the textile industry and many museums adopted them for its use

20 Browne 1884, p. 66.

21 Substances with and over 0.3% arsenic in mass content are banned in Germany. The production of DDT is forbidden and the use of mercury compounds is strictly reduced to limited uses. GefSoffV, Anhang IV, Nr. 3; Nr. 7; Nr. 20 (since 2010 added to the GefSoffVuaÄndV). https://www.buzer.de/gesetz/6621/a94226.htm (22. 01. 2022)

22 The use of arsenic in the USA, UK and Netherlands occurred until the 1980s. Bacon et al. 2011, p. 113. However, the use of arsenic trioxide remained popular through the following years and it is known between preparation technicians that old batches are still used in some cases. The last documented application in the Zurich region (Switzerland) took place in 2017. Urs Näf, Kantonales Laboratorium Zürich, pers. comm. 2018.
One of the authors had the chance to preserve a bird mount during an internship in the Museo Nacional de Historia Natural (MNHN) in Santiago de Chile in the year 2014, where a mixture of arsenic trioxide as a powder was applied directly with a brush inside of the skin for a "effective and long-lasting" preservation. The use of arsenic trioxide at the MNHN was banned in 2018. Richard Faundez, MNHN, 2022, pers. comm.

23 The most popular Eulans in German speaking countries used to treat tanned skins of specimens were Eulan U33 (widely used until 2000) and Eulan SPA 01 (used as an alternative, since the discontinuation of the manufacture of Eulan U33). Feuersenger 2018, p. 39. Eulan U33 contains chlorphenylid, a mixture of PCSD and PCAD, which are qualified as medium to highly toxic environmental chemicals of predominantly high persistence like DDT and lindane. Homolka 2015(II), p. 162. A good overview and history of other Eulans can be seen in Homolka 2015(I) pp. 19–30.

in taxidermy.[24] A major problem is that many independent taxidermists, institutions and museums purchased large quantities of this product at that time, as well as arsenic trioxide and other hazardous substances. Therefore, the application of these pesticides is still possible despite the expired date of manufacture and the prohibition of these substances. Some of these hazardous chemicals are still recommended in professional literature.[25]

Formaldehyde has been classified as "may cause cancer" since 2014 in Germany[26] (Category 1B according to CLP Regulation) and is one such problematic example. After the discovery of its preservation properties at the beginning of the 20th century, this substance began to replace the use of ethanol for the manufacturing of fluid preserved specimens. The advantage of formaldehyde (as an aqueous solution) is that it works as a fixative and evaporates much more slowly than ethanol.[27] It is still found in many preservation fluids today as the main hazardous substance.[28] Another hazardous substance frequently used in fluid preservation was phenol. It had widespread use in medical preparation, especially in German speaking countries. It was a compound of many fluid mixtures used to preserve anatomical collections and was formerly called carbolic acid.[29]

Some preparation technicians in Swiss natural history museums have been searching for an alternative, like biocide-free preservation methods, since the 1990s. The need for biocide-free taxidermy has been especially discussed since the implementation of the "Biocidal Products Regulation" (BPR, Regulation (EU) 528/2012) of 2012. In 2017 a conference of the Federation of Natural Science Preparators of Switzerland (VNPS) was held in St. Gallen to discuss the impact of the new legislation in Swiss museums, since a lot of the typically used biocides where being banned.[30] At the end of the same year in Geneva the VNPS was the first professional association to offer a

24 e.g. Tello/Paz 2013, p. 10. This replacement took place mostly in German speaking countries and was spread through the book *Makroskopische Präparationstechnik. Teil 1: Leitfaden für das Sammeln, Präparieren und Konservieren: Wirbeltiere,* edited by Rudolf Piechocki (1919–2000), first published in 1961. This compilation of different preparation methods was revised and updated five times until 1998 (in that year with his colleague H. J. Altner), becoming one of the most important books about preparation technology written in German, especially because of its extensive bibliography related to the preservation and management of natural history collections.

25 e.g. Piechocki/Altner 1998, p. 24 (use of arsenic trioxide); p. 28 (use of naphthalene and PDCB); p. 123 (use of lindane); pp. 194–195 (use of carbon tetrachloride, trichloroethylene, hydrogen cyanide and phosphine among other highly hazardous substances, but advising to follow local regulations).

26 https://www.umweltbundesamt.de/themen/gesundheit/umwelteinfluesse-auf-den-menschen/chemische-stoffe/formaldehyd#was-bedeutet-krebserzeugend (03. 01. 2022).

27 Ethanol is often described as a "pseudo-fixative". e.g. Simmons 2014 p. 26.

28 e.g. Simmons 2014, p. 33; König/Winter 2020, pp. 15–16.

29 e.g. Piechocki/Altner pp. 321–322; Steinmann 1982, p. 181.

30 Troxler 2017.

workshop in biocide-free taxidermy. It was demonstrated that even small bird skins can be preserved using only tanning methods, avoiding the use of biocides.[31] There are also new approaches in fluid preservation for enhancing work safety by replacing preservation fluids with nonflammable and nontoxic substances like glycerol.[32] Combined with new care strategies for collections as IPM (integrated pest management) and improved concepts for preventive conservation, the opportunities to create new, biocide-free specimens seem not only possible, but mandatory.

Since hazardous substances were used to preserve natural history collections until the end of the 20th century, it can be assumed that all of these collections are contaminated. This has not been fully comprehended by many institutions to date. As a result, many museums lack awareness and strategies for dealing with contaminated collections, as well as a concept for a potential remediation plan. In many cases preparators are even wrongly encouraged by collection managers, curators and specialist literature to use harmful substances for the preservation of natural history collections and specimens.[33]

Biocides on dry preserved specimens can be detected non-destructively with X-ray fluorescence (XRF). This procedure is a quick method to determine if traces of arsenic, lead or mercury are present.[34] The measured values provide only a qualitative result, which means that the sample is contaminated but they do not indicate the amount of contamination.[35] Reliable information for more complex synthesized substances may require more specific analytical instruments, as well as appropriate experience in interpreting the results.[36] Raman spectrometry and gas chromatography-mass spectrometry (GC-MS) are methods that provide accurate information about the composition of preservation fluids.[37] Simpler methods for testing traces of formaldehyde in the preservation fluid are sodium sulfite titration or formaldehyde test strips.[38] Other useful sources about the composition of the preservation fluid are specimen associated publications.

The health risk for museum professionals working with dry specimens is usually not the direct contact with arsenic or mercury but rather indirect exposure. The dust produced by the specimens, consisting of accumulated particles of hair, feathers, skin,

31 Lunak 2020, p. 60.

32 e.g. Van Dam 2020.

33 The use of old batches of Eulans or Borax (banned in Germany since 2009) is still common in some German speaking institutions since it has a high efficacy and saves time (and therefore money) in monitoring the collection for longer periods. e.g. Feuersenger 2018. pp. 39–41.

34 e.g. Odegaard et al. 2006; Bacon et al. 2011; Seewald 2017.

35 Bacon et al. 2011, pp. 111–112.

36 e.g. Paz/Behrendt 2020. pp. 28–29.

37 e.g. Cersoy et al. 2020, pp. 54–56.

38 e.g. Finkelde/Waller 2020, pp. 33–35.

etc., is such an indirect factor.[39] Air currents, like the ones caused by vacuum cleaners, can stir up this dangerous dust, which can cause health problems if inhaled. But even if the dust is stirred up, it tends to fall quickly to the ground due to its high molecular weight, reducing the likelihood of inhalation.[40] Specific hygiene regulations would be an effective prevention strategy in the museum collection storage, with the goal of removing dust frequently. However, it is necessary that the staff carrying out this work is aware of this risk and is trained accordingly.

When working with natural history collections, it is mandatory to consider occupational safety. This means that personal protective equipment (PPE) should be adapted to the specific situation.[41] In the case of working with dry specimens, subdividing areas to create specific working stations can improve the health safety. The areas should consist of a decontamination and a hygiene workstation. The first one is used to deal with the contaminated material and the hygiene workstation is spatially separated and used for administrative work.[42] In addition, a “gray” workstation could be defined, where contaminated material is stored and work on cleaned specimens can be performed, as long as the dust production can be excluded.[43] It should be ensured that no phones or computers are used in the decontamination workstation to minimize the spread of hazardous substances. The different stations should be separated by a sluice, so that contaminated work clothes and tools can be changed and cleaned in this area, thus avoiding the transfer of hazardous material.

Another important measure is the risk assessment for the staff. This can include biomonitoring of people working with contaminated specimens. In this way, an accurate diagnosis can be made in case of health issues.[44] Pregnant and breastfeeding women in particular have, in some countries, a high labor law protection status and are not allowed to work with hazardous materials.[45] Therefore it is of high importance to consult and implement the local regulations. This also applies to employees, who are obliged to accept and follow the measures. The air quality of the working area should

39 e.g. Leimbrock/Wagner 1998 pp. 115–116; Ammann/Leu 2019 p. 15.

40 Probst et al. 2019. pp. 16–17.

41 The proper PPE depends on the type of collection material being worked with. For example, FFP2 masks are necessary when handling collections that may detach hazardous dusts, but will not help when working with gases released by formaldehyde solutions. For fluid-preserved collections, fume hoods or gas masks are needed for safe handling. e.g. Burroughs et al. 2006, p. 53.

42 This concept is derived from the “black and white workstation concept” used for the work with contaminated material. e.g. P-Dekon NRW 2011, p. 14.

43 This only applies to work steps that don’t produce dust, for example coloring, modelling or working with very fine tools.

44 Spiegel et al. 2019, p. 52.

45 ibid. p. 85.

be regularly monitored and adjusted to the current legal regulations, otherwise the local authorities may prohibit access to the workplace.[46]

There is already some experience with possible decontamination techniques for cultural heritage using supercritical carbon dioxide.[47] For natural history or anatomical collections, there are no reliable publications that have effectively applied this method. To close this gap, a Swiss research project has planned to investigate the decontamination method using supercritical carbon dioxide on natural history specimens. Due to the lack of proper funding and lobbying for this kind of research, there are unfortunately still no results available.[48]

Natural history and human anatomical collections are important pieces of cultural heritage. Therefore, it is important to consider some ethical conservation principles when dealing with these kinds of collections. It is of major concern to consider the specimens in their entirety, including added materials. This means that pedestals, jars, labels, wires, fillings and even old repairs are part of their history. They play an important role in its cultural significance.[49] To achieve this, one of the primary premises for any kind of treatment is to establish a clear function of the specimen or collection. The following questions can be helpful in this process:

- What is the significance of the specimen or collection today?
- Can an ethical handling be assured?
- Should it be exhibited or not?
- What do we know about its provenance?
- Is there any evidence that preservation cannot be adequately ensured?

In this manner, it is possible to formulate an appropriate treatment and implement a strategy to achieve a better conservation of a specific specimen or collection. This should be managed by the professional staff such as preparation technicians and specialized conservators. Consequently, it is of mayor importance to promote interdis-

46 This applies specially to hazardous substances as formaldehyde. For example, in Germany at the Ruhr University in Bochum, the anatomical preparation course was closed for over a year due to new policies about formaldehyde values in the air, which were lowered between the years 2015 and 2016. This lowering of levels caused a complete shutdown of the activities in the dissection room, costing some of the students more than three semesters to make up for the lost practice. https://www.spiegel.de/lebenundlernen/uni/bochum-medizinstudenten-wollen-nicht-auf-leichen-verzichten-a-1099553.html (03. 01. 2022).

47 e.g. Unger et al. 2011.

48 A group of researchers from the University of Applied Sciences Biel, the Swiss National Museum and the Natural History Museum of the Bern (NMBE) are involved in the project.

49 “Cultural significance means aesthetic, historic, scientific, social or spiritual value for past, present or future generations.”. ICOMOS 2013, p. 2. The term “significance” is also used to describe the communicative mechanisms as well as the symbolic nature of cultural heritage, e.g. Russell/Winkworth 2009.

ciplinary cooperation between these two professions, which are responsible for the preservation of natural history and anatomical collections.

Professional associations play an important role in this whole issue, especially in the handling of hazardous substances in collections. They act as networking organizations, providing support and advice to their members and are the main point of contact when information for specific concerns about legal issues is needed. Additionally, they provide the opportunity for proactive work with authorities and creating an appropriate lobby for research on issues regarding the care of cultural heritage.[50] Therefore professional associations should establish safety standards, promote networking and knowledge transfer between specialists and other associations. In Switzerland there is already a very fruitful collaboration between the VNPS and the Association of Swiss Restorers and Conservators (SKR). It enabled a restoration project of the historic natural history collections at the Einsiedeln Abbey in Switzerland from the late 18th century.[51] This cooperation can be seen as an exemplary project between preparation technicians, conservators and restorers.

It is necessary to exchange knowledge and expertise between specialists of related subjects to achieve the accurate preservation of our cultural heritage, which should be a common goal of all museum professionals.

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50 e.g. Ammann/Leu 2019.

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